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(71) Applicant *(for all designated States except US)*: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 2199 Addison Street, Berkeley, CA 94720 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : VAN GUNDY, Seymour, D. [US/US]; 4731 Chicago Avenue, Riverside, CA 92507 (US). ZAVALETA-MEJIA, Emma [US/US]; 1789 7th Street, #213, Riverside, CA 92507 (US).

(74) Agent: ROWLAND, Bertram, I.; Townsend and Townsend, One Market Plaza, Steuart Street Tower, San Francisco, CA 94105 (US).

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(57) Abstract

Rhizobacteria having nemastatic properties introduced to the rhizosphere of a plant host are to protect said plant host against nematode and fungal infestation. Particularly, *Serratia marcescens* strain NA1 provides significant protection to susceptible plant hosts and can be used as a source for genetic material for introduction to other bacteria to provide the desired properties. Colonization of the rhizosphere and production of anti-nematodal toxins by the rhizobacterial strain may be enhanced by the application of a suitable nutrient source, particularly one having assimilable nitrogen.

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RHIZOBACTERIAL PLANT PROTECTION

The present application is a continuation-in-part of U.S. application serial no. 545,722, filed on October 26, 1983.

BACKGROUND OF THE INVENTION

Field of the Invention

Nematodes and fungi remain a serious problem in the cultivation of crops. The presence of these pests results in reduced crop yields and necessitates substantial expenditures in employing chemical biocides. In some crops, it is found that resistant cultivars have lower yields than susceptible cultivars. It is therefore of particular interest to find means for reducing infestation, particularly by natural ways.

Description of the Prior Art

Zavaleta-Mejia and Van Gundy, Journal of Nematology (1982) 14:475A (Abstract) describes the effects of rhizobacteria on Meloidogyne infection. The application of rhizobacteria to plants to inhibit the growth of harmful bacteria is known. See, e.g., Kloepper et al. (1981) Phytopathology 71:1020-1024; Kloepper et al. (1980) Nature 286:885-886; and Kloepper et al. (1980) Curr. Microb. 4:317-320. The effect of applying a nematocide (aldicarb) on the bacterial population in the soil-root interface of tomato plants was reported by Zavaleta-Mejia and Van Gundy (1984) J. Nematol. Abstract No. 295 (Presented at First International Congress of Nematology, Guelph, Ontario, Canada, August 1984).

SUMMARY OF THE INVENTION

Rhizobacteria are provided for protection against nematode and fungal infection. Rhizobacteria which are selected as being antagonistic to growth of nematodes can be used to inoculate seeds and plant parts to provide the protection. The rhizobacterial

strains are not toxic to seed germination and are able to colonize the roots of the plant during and after germination. To enhance growth of the bacterial strains, as well as promote the production of anti-nematode toxins, the rhizobacteria may be applied to the rhizosphere in the presence of an assimilable nitrogen and carbon source.

DESCRIPTION OF SPECIFIC EMBODIMENTS

Rhizobacterial strains are employed to inhibit nematode and fungal infection of susceptible plant hosts. Selected rhizobacterial strains are inoculated on the seed or seeding or in the soil at the time of planting, preferably in combination with a nitrogen nutrient source to promote initial colonization of the rhizosphere. Supplemental applications of the rhizobacteria may be performed from time to time, conveniently in conjunction with crop irrigation.

Bacteria suitable for use in the present invention are characterized by (1) their ability to inhabit the rhizosphere of the plant host being treated, (2) lack of toxicity to the plant host and other beneficial soil microorganisms which inhabit the rhizosphere (e.g., nitrogen-fixing soil bacteria such as rhizobium), and (3) their ability to inhibit the infection of pathogenic nematodes, and (4) promote growth of the plant host. Suitable rhizobacteria can be selected by screening a bacterial population indigenous to the plant host of interest for bacteria demonstrating nematocidal and fungicidal activity, as well as a lack of toxicity to seed germination. Such screening may be accomplished by growth of isolated rhizobacterial strains in a nutrient medium, separation of the rhizobacteria from the nutrient medium and use of the nutrient medium (which may be supplemented with additional nutrients), as a nutrient strain for the rhizobacterial production and nematode toxins. Reduced

growth of the pest as compared to a control employing the same medium, except one that was not conditioned with the rhizobacteria, is indicative of nemastatic properties. This screening technique may be used to identify nemastatic rhizobacteria from a variety of bacteria, including Serratia, such as Serratia marcescens, Pseudomonas, such as Pseudomonas fluorescens and Pseudomonas putida, Bacillus, such as Bacillus subtilis, and Actinomycetes.

The present invention may be used with virtually any plant which is subject to nematode and fungal infestation, particularly crop plants including both vegetables and fruits. Specifically, the rhizobacteria may be used to inhibit nematode and fungal infestation with such crops as sugar beet, beet, tomato, cucumber, cabbage, beans, soybeans, cotton, strawberries, celery, potatoes, and the like.

Pests which may be controlled by the present invention include plant parasitic nematodes such as root knot nematodes and cyst nematodes. Fungi which may be controlled include such fungus as Fusarium, Rhizoctonia, Pythium, Verticillium, and Phythophtora.

Treatment will involve introducing the selected rhizobacterial strain to the rhizosphere of the plant host to be protected, preferably at least as early as the time of planting. The rhizobacterial strain may be applied to either a plant part or the soil, conveniently as a dispersion or suspension, inoculum, in powder or in granules, or the like, having a sufficient amount of the rhizobacteria and nutrient starter to provide protection. The manner of application is not critical. However, the timing is critical. The rhizobacterial strain must be active and growing when the plant roots enter the soil to provide early protection from nematode infection. Additional applications following planting through irrigation provide continuing protection. Plant parts which may

be treated include seeds, seedlings, plantlets, propagules, tuber portions or sections, and the like.

The plant part may be initially inoculated by dipping in or spraying with a suspension containing the bacterium, desirably at a concentration of at least 10^4/ml, usually in the range from about 10^5/ml to 10^9/ml. Bacterial concentration may be measured conveniently in a Spectronic 20 spectrometer at a wavelength of 600 nm. For the exemplary Serratia marcescens strain NA1, the desired concentration corresponds to an absorption greater than about 3% light absorption, preferably greater than about 4% light absorption, more preferably about 4% to 12% light absorbance. While water may be used as the suspending agent, the suspending agent is preferably a nutrient starter broth. The plant seed can be dipped into the suspension, drained free of the suspension and allowed to dry before planting for germination.

It will often be desirable to continue the application of the selected rhizobacterial strain after the plant host has been planted. Such subsequent treatment helps assure continued vigorous colonization of the rhizosphere by the nemastatic rhizobacteria. Subsequent applications should be carried out at least once during the growing season, preferably twice, and as frequently as once a week. Conveniently, application may be accomplished in conjunction with watering and/or fertilizing. Applying the rhizobacteria by means of a drip irrigation system has been found to be a particularly effective manner of introducing the rhizobacteria to the rhizosphere during the growing season.

While the rhizobacteria may be used by themselves, they can conveniently be used in conjunction with a chemical pesticide specific for the pest (but non-toxic to the rhizobacteria). Therefore, in addition to the use of the rhizobacteria, the plants

may be exposed to a nematocid in accordanc with the condition specified for such chemical pesticides.

For initial application of the selected rhizobacteria to the rhizosphere, it is desirable to include a suitable nutrient source to enhance colonization of the soil by the rhizobacteria and promote the production of various substances toxic to nematodes and fungi, particularly gaseous and non-gaseous ammonia. The nutrient source should include carbon and assimilable nitrogen, since many soils will be relatively nitrogen poor. Suitable nutrient sources include organic sources having available amino groups, usually proteins and polypeptides such as peptones. Also suitable are various nutrient starter broths, such as Bacto Nutrient Broth from Difco. A particular peptone which has been found suitable is available from Sigma Chemical Co., St. Louis, Missouri, as Type 1 peptone (No. P7750).

The following examples are offered by way of illustration and not by way of limitation:

EXPERIMENTAL

Methods and Materials:

Screening - Rhizobacterial cultures were screened for the ability to reduce root galling caused by the root knot nematode (Meloidogyne incognita) and the ability to promote the growth of tomatoes (cv Tropic) and cucumber (cv Spacemaster) in a naturally infested grape soil contained in polystyrene cups. Seeds were germinated in vermiculite and at 10-11 days were dipped in a bacterial suspension (9% light absorbance at 600 nm on a Spectronic 20) and then planted in the soil. After 4 weeks, the dry weight of the shoot and gall index of the root were determined and given an index as compared with the control.

Field testing - A number of selected rhizobacteria strains were chosen for field testing. These cultures are given in Table 1 with growth stimulation

and nematode indexes. Three fields were selected for testing. Field 9E was located on the Riverside Experiment Station and is infested with the sugar beet cyst nematode (Heterodera schachtii). Crops to be planted were cabbage and sugar beets both hosts to this nematode. The field was planted on April 29, 1982. The soil temperature was 20°C. The field was sprinkler irrigated twice a week. Soil texture was sandy loam. Field S.C. was located at the South Coast Field Station in Irvine, California and is infested with the root knot nematode, Meloidogyne incognita, and has been cropped to vegetables for several seasons. This field has always been considered an excellent field for nematode damage. Crops planted were sugar beet, tomato and cucumber. The field was planted on May 27, 1982. The soil temperature was 22°C. The field was irrigated by drip irrigation twice a week. Soil texture was sandy loam. Field 4B was located on the Riverside Experiment Station and had previously been cropped to figs which were heavily infested with the root knot nematode, Meloidogyne incognita. Crops planted were sugar beet, tomato and cucumber. The field was planted on June 16, 1982. The soil temperature was 24°C. The field was irrigated by furrow irrigation. Soil texture was Hanford coarse sandy loam.

The germinated and inoculated seeds were planted in Speedling trays filled with a peat moss-vermiculite mix with pH adjusted to 6.7. After the seedlings were about 3 inches tall, they were removed to the lathhouse for hardening and inoculation. Two days before transplanting in the field, the individual trays were watered with their respective bacterial suspension prepared in deionized water to a 9% light absorbance on a Spectronic 20 spectrometer at 600 nm. The seedlings and root balls were transplated in the field using care to avoid contamination from one lot to

the other. The fields received regular agricultural practices throughout the growing season.

The cabbages were harvested in three picks by removing solid heads and weighing. Cucumbers were harvested weekly for 3 harvests. Collected fruits were weighed. Tomatoes were harvested at one pick by weighing all fruit on the vine. Sugar beets were topped and beets weighed. Data was summarized on the basis of yield per plant. Roots were dug at final harvest and rated for root knot nematode galling using a standard index 0-5 with 5 being 100% galled. Soil samples were taken for analysis of dried cysts.

Table 1: Summary of the results obtained from greenhouse screening tests of the rhizobacteria chosen for 1982 field trials.

Bacteria	% Decrease in G.I.		% Increase in FWI	
	Tomato	Cucumber	Tomato	Cucumber
1A6	-12	-11	25	36
5A4	-8	-6	38	27
5A5	-5	-4	44	18
5D3	-19	-9	43	32
6D20B	-19	-8	28	27
HB2	-49	0	25	4
JC15	-9	-34	0	15
NA1	-67	-30	12	6

G.I. = % increase or decrease in nematode root galling index of 0-5 where 5 is 100% of root system galled.

FWI = % increase or decrease in fresh weight of total plant after 30 days.

Soil temperature = 25-28°C.

Table 2: Summary of field trials of rhizobacteria on growth and nematode galling of tomatoes.

Isolates	FIELD LOCATION S.C. F.W.I.	S.C. G.I.	4B F.W.I.	4B G.I.
NA1	-9	-21	+27	-23
6D20B + 5D3	+5	-15	-19	-29
5D3	+3	-41	-7	+3
JC15 + HB2	-25	-5	+26	-20
JC15 + 5A5	+6	-14	+60	-26
34R + SH5RN	-20	+5	+92	-34

FWI = % increase or decrease in yield of tomato fruit on a single pick.

G.I.= % increase or decrease in nematode root galling index.

Table 3: Summary of field trial of rhizobacteria on growth and nematode galling of cucumbers.

	FIELD LOCATION			
	S.C.		4B	
Isolates	F.W.I.	G.I.	F.W.I.	G.I.
NA1	-21	0	+19	-31
6D20B + 5D3	+21	0	+31	-36
5D3	+17	-20	+25	-2
JC15 + HB2	-33	+60	+97	-3
JC15 + 5A5	-20	+30	+48	-38
B4R + SH5RN	-2	-10	+126	+2

FWI = % increase or decrease in yield of cucumbers on a single pick.

G.I.= % increase or decrease in nematode root galling index.

Table 4: Summary of field trials of rhizobacteria on growth of nematode galling of sugar beets.

Isolates	FIELD LOCATIONS 9E F.W.I.	9E G.I.	S.C. F.W.I.	S.C. G.I.	4B F.W.I.	4B G.I.
		1/		2/		2/
NA1	+5	-	+1	-12	+34	-9
6D20B + 5D3	-12	-	+41	-5	+23	-65
5D3	-32	-	+24	-10	-7	-54
JC15 + HB2	-11	-	+25	-1	-1	-36
JC15 + 5A5	-6	-	+20	-10	+21	-52
B4R + SH5RN	-6	-	-3	-3	-14	-61

FWI = % increase or decrease in fresh weight of total plant after 30 days.

G.I.= % increase or decrease in nematode root galling index.

1/ Sugar beet cyst nematode, Heterodera schachtii (no galling).

2/ Root knot nematode, Meloidogyne incognita.

Table 5: Incidence of root rot in sugar beets at South Coast Field Station.

Cultures	% Rotted Beets
6D20B + 5D3	20
5D3	28
JC15 + HB2	35
JC15 + 5A5	16
NA1	29
B4R + SH5RN	42
C ntrol	52

In the next study, the effect of bacterialization alone and in combination with Temik, a nematocide, upon root-knot infection on the yield with two tomato cultivars was studied.

Materials and Methods:

Groups of seeds of two different tomato cultivars, 7718 VF hybrid and VFN bush, were immersed in bacterial suspension (4% of transmittance), NA1, HB13 or a mix of both (bacteria were 48hr old), or in water for 5min. Then the seeds were sown in plastic trays filled with vermiculite, one seed per hole. A small amount of fertilizer was placed in each hole of the plastic tray. A second bacterial inoculation was made after 21 days, 4ml of the corresponding bacterial suspension or mix of bacterial suspensions was placed in every hole of the tray. Trays were maintained in the greenhouse.

When the seedlings were 28 days old they were transplanted (May 28th) in a field infested with root-knot nematodes. The amount of nematocide, Temik (granular 15%), applied was 6.3g/plot (3lbs a.i./acre). Each treatment (16 total) had 5 replicates, each one was a row 12' long containing 12 plants. The experimental design was a randomized block.

Results:

The experiment was evaluated on August 2nd. In each replicate the tops of the plants together with fruit were weighed. Also 5 root systems were dug and the gall index was rated using a scale from 0 to 5. The results obtained are summarized in Table 6.

Table 6: Yield and gall index obtained from a r ot-knot susceptible and a root-knot resistant cultivar treated with two rhizobacteria and Temik alone and in combination.

Treatment	Yield[a] (Fruit and Shoot) Kg	Gall Index[a]
Susceptible cv.		
Control	28.9 AB	3.7 A
Control + Temik	31.9 AB	4.0 A
NA1	36.3 A	3.2 A
NA1 + Temik	25.6 B	3.2 A
HB13	31.8 AB	3.2 A
HB13 + Temik	24.6 B	4.4 A
NA1 + HB13	23.5 B	4.0 A
NA1 + HB13 + Temik	28.2 AB	3.5 A
Resistant cv.		
Control	28.3 AB	0 B
Control + Temik	27.6 AB	0 B
NA1	30.7 AB	0 B
NA1 + Temik	28.1 AB	0 B
HB13	28.1 AB	0 B
HB13 + Temik	28.5 AB	0 B
NA1 + HB13	25.5 AB	0 B
NA1 + HB13 + Temik	25.0 B	0 B

[a] Average of 5 replicates. Values in each column followed by the same letter(s) do not differ significantly (p = 0.05).

The following conclusions were drawn from the above data. The control of the susceptible cultivar gave the same yield as the control of the resistant one (no root-knot infection at all was observed), even though the former was severely galled by the nematode. This confirms that resistant varieties are usually less productive than susceptible ones.

Treatment with Temik alone or with one bacterium only always increased yield over the control (from about 10 to 25%) in the susceptible cultivar. No increase was obtained with the same treatment in the resistant cultivar, except with NA1 which gave an increase of 8.5%. This suggests that plant growth is increased and somehow the root-knot infection has been affected by the presence of the bacterium. While various explanations might be given for the observation, support for the fact that the bacterium directly affects the nematode is obtained in in vitro tests which indicate that NA1 affects the motility of the nematode.

There was no observed correlation between yield and the degree of galling. One explanation for this inconsistency is that the method for nematode control applied (chemical or biological) was most effective only for a short time (3-4 weeks after planting), and after the protected period, nematode infection occurred. Therefore, at the end of the season, almost the same degree of galling was observed in all the treatments, masking the protective early effect of the method of control applied at the beginning of the season, which was only expressed by the increasing yield obtained. Therefore, it is necessary to make continued applications of the rhizobacteria during the growing season to maintain continued protection from the nematodes.

In general, combinations of bacteria or bacteria with nematocide had no positive or synergistic

effect on th plant. Rather, the observation was that in most cases the effect upon the plant was negative and a decreased yield was obtained.

In another study, in vitro experiments and greenhouse experiments suggested that NA1 suppresses migration of root-knot nematode larva by as much as 70%. In other tests it was demonstrated that NA1 significantly reduced Fusarium oxysporum f. lycopersica infection of susceptible tomato plants grown in greenhouse pot experiments. Tests to improve the inoculation procedure demonstrated that nutrient broth-bacteria dips were more effective in reducing root-knot infection than washed-bacteria dips. The nutrient starter was not toxic to the nematodes until bacterial cells were added to the test system. Both volatile and water soluble toxins to the nematodes were formed by the bacteria growing on the nutrient starter. Tomato seed inoculation tests indicated that NA1 is not toxic to seed germination.

Based on the above results, it was concluded that the culture NA1 appears to be a promising rhizobacterium with both nemastatic and fungistatic properties. NA1 is representative of other rhizobacteria which may be used in place of NA1, where such bacteria have the same properties.

The following experiments demonstrated that the application of a nitrogen nutrient source together with the rhizobacteria serves to enhance the nematocidal effect. Subsequent tests both in the greenhouse and in the field have shown that peptone, a major ingredient of the nutrient broth and agar can also stimulate indigenous fluorescent rhizobacteria and can enhance nematode supression in some soils.

Table 7: Effect of different S. marcescens NA1 preparations and nutrients on root-knot infection in field soil.

Treatments	Gall Index	Dry Weight of the shoot (g)
Nematode (N)	100.00 ± 0.00 A	0.004 ± 0.01 A
S. marcescens NA1-cells[a] + N	98.75 ± 2.31 A	0.042 ± 0.07 A
S. marcescens NA1-filtrates[b] + N	81.40 ± 16.20 B	0.299 ± 0.20 B
Nutrient agar-washings[c] + N	77.50 ± 22.30 B	0.316 ± 0.28 B
Broth 80 mg/pot[d] + N	49.00 ± 24.74 C	0.562 ± 0.25 C
LSD (P = 0.5)	16.8	0.196

Figures represent the average of eight (8) replications. Means in each column followed by a common letter are not significantly different according to Duncan's multiple-range test (P = 0.05). Two seedlings per pot (350ml styrofoam cups) were transplanted. Seedlings were 10 days old. Each pot was inoculated with 5,400 nematode larvae. Bacterium was grown for 48 hours at 29°C.

a Cells suspended from the Petri dish cultures in distilled sterile water were centrifuged at 6,000 rev min^{-1} for 15 min and then resuspended in distilled sterile water. (10^9cfu/ml).

b The supernatant from [a] was passed twice throughout a filter membrane (0.2 μm), and the resulting filtrate was used as inoculum.

c Petri dishes containing nutrient agar were flooded with distilled sterile water (as it was done with the plates containing the bacterium), and after 30 min the water was removed from the plates and used as inoculum.

d Eight grams of Bacto Nutrient Broth (Difco) were dissolved in 1L of distilled water and autoclaved at 15 p.s.i. at 120°C for 15 min. Twenty ml of this broth medium were applied in each pot.

Peptone Type 1 (No. P7750, Sigma Chemical Co.) was added to two different types of soil (field soil and pasteurized sand) in 4 inch pots. The peptone was added in water to the concentration shown in Table 8, followed by the addition of root gall nematodes (200 to 500/pot). Two week old tomato plants (cv. tropic) were then transplated to each of the cups. Prior to planting, the seedlings were treated with NA1 in the manner described above. After 4 weeks, the tomato plants were removed and assessed for Gall Index and Dry Weight. The results, as set forth in Table 8, clearly indicate that substantial growth enhancement and gall index reduction is achieved by the addition of the Peptone nutrient starter to the field soil having a normal bacterial background.

Table 8: Effect of Peptone on Root-knot Infection in two kinds of soil.

Treatments	Gall Index[a]	Dry Weight Shoot[a]
Field Soil		
Nematode (N)	80.12 A	0.62 C
Peptone 1 ppm + N	64.14 B	0.69 BC
Peptone 10 ppm + N	53.28 BC	1.04 ABC
Peptone 100 ppm + N	43.50 CD	1.44 A
Peptone 1,000 ppm + N	46.00 CD	1.16 AB
Peptone 10,000 ppm + N	32.00 D	0.90 BC
Pasteurized Sand		
Control	0.00 C	2.41 A
Nematode	52.87 AB	2.35 AB
Peptone 1 ppm + N	59.50 A	1.97 B
Peptone 10 ppm + N	60.75 A	2.04 AB
Peptone 100 ppm + N	46.62 AB	2.01 AB
Peptone 1,000 ppm + N	43.50 B	2.08 AB
Peptone 10,000 ppm + N	12.83 C	1.08 C

[a] Average of 5 replicates. Values in each column followed by the same letter(s) do not differ significantly (p=0.05).

The ability of a nitrogen nutrient starter, such as peptone, to enhance bacterial growth without a concurrent enhancement of fungal growth is shown in Table 9. Root gall nematodes and either peptone or a nutrient broth were added to pots of field soil. The growth of bacteria and fungi in soil subjected to each treatment was then measured on selective media after 12 hours. The results demonstrate that the nitrogen nutrient starter enhances the bacterial growth particularly the fluorescent pseudomonas group, without substantially affecting the fungal growth.

Table 9: Effect of Peptone and Broth on the Soil Bacteria and Fungi Population.

Treatment	Bacteria[a,b] (c.f.u. per gram of dry soil x 10^9)	Fungi[a] (c.f.u. per gram of dry soil x 10^9)
Control (nematode only)	81.27 ± 8.10 D	6.69 ± 1.58
Peptone (52mg/pot) + nematode	117.67 ± 7.23 C	4.77 ± 0.81
Peptone (120 mg/pot) + nematode	189.10 ± 5.62 B	6.69 ± 0.87
Broth (120 mg/pot) + nematode	235.75 ± 26.31 A	7.55 ± 3.53

[a] Each figure represents the average of 4 replicates except in the treatment Broth 120 mg/pot + nematode which had only 3 replicates.

[b] Bacterial growth was measured on three selective media, and the total of the three figures is reported in this Table.

NA1 can be used in a variety of ways for obtaining bacteria as seed inoculants, which can provide for the fungistatic and nemastatic properties of NA1. By inducing production of the nemastatic and fungistatic secretory product, one can hybridize the messenger RNA which is produced against a rhizobacterium of the same species which does not provide for nemastatic or fungistatic properties. Similarly, one could obtain the messenger RNA from a rhizobacterium which does not have such properties and hybridize it to the genome of the rhizobacterium which does. The messenger RNA would not hybridize to the genomic DNA of the rhizobacterium lacking the biostatic properties and the genomic DNA of the rhizobacterium which has biostatic properties, which does not

hybridize to the messenger RNA from the rhizobacterium which does not have biostatic properties, may then be screened for coding for products which have biostatic properties. It may prove that a single gene does not encode for the product which has the biostatic properties, in which case there would have to be substantial screening of the rhizobacterium having the biostatic properties to establish the genes necessary for imparting biostatic properties to a different host. However, one can use the genomic DNA or messenger RNA for screening the genomes of rhizobacteria to determine whether they are candidates for providing biostatic characteristics.

Where one or more genes have been established as the required genes for producing a biostat, these genes may be inserted into appropriate vectors which provide for expression of the genes and biostatic properties imparted to a host which may have other desirable properties, such as good growth, strong competitiveness in the field, or the like.

In accordance with the subject invention, rhizobacteria are provided which provide protection from nematode and fungal infestation. By inoculating seed with the biostatic rhizobacteria, one can substantially reduce infestation while enhancing yield.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

The rhizobacterial strain NA1 was deposited at the American Type Culture Collection, Rockville, Maryland, U.S.A., on October 21, 1983 and given Accession No. 39473.

WHAT IS CLAIMED IS:

1. A method for inhibiting nematode and fungal infestation of a plant host, which method comprises introducing into the rhizosphere of the plant host a rhizobacterial strain which has been selected as being antagonistic to the infection of a plant parasitic nematode.

2. A method according to Claim 1, wherein the rhizobacterial strain is introduced to the rhizosphere by inoculation of a seed for the plant host prior to planting.

3. A method according to Claim 1, wherein the rhizobacterial strain is introduced to the rhizosphere by inoculation of the soil prior to planting the plant host therein.

4. A method according to Claim 2, wherein said seed is inoculated by dipping the seed into a nutrient broth suspension of said rhizobacterium.

5. A method according to Claim 1, wherein the rhizobacterial strain is introduced to the rhizosphere subsequent to planting the plant host.

6. A method according to Claim 5, wherein the rhizobacteria is introduced by means of a drip irrigation system.

7. A method according to Claim 1, wherein the rhizobacteria is introduced to the rhizosphere together with a nutrient source including assimilable nitrogen.

8. A method according to Claim 7, wherein the nutrient source is a peptone or a nutrient broth.

9. A method according to Claim 1, wherein the rhizobacteria strain is selected from the group consisting of Serratia, Pseudomonas, Bacillus, and Actinomycetes.

10. A method according to Claim 9, wherein the rhizobacteria strain is of the species Serratia marcescens.

11. A method for protecting a host plant from nematode and fungal infestation, which method comprises:

inoculating a seed or a seedling of said host plant with the rhizobacterium NA1 prior to planting.

12. A method according to Claim 11, wherein said seed or seedling is inoculated by dipping the seed or seedling into a nutrient broth suspension of NA1.

13. A method according to Claim 11, wherein said seed or seedling is cucumber seed or seedling.

14. A method according to Claim 11, wherein said seed or seedling is tomato seed or seedling.

15. A method according to Claim 11, wherein said seed or seedling is a beet seed or seedling portion.

16. A method according to Claim 1, further comprising the step of applying a nutrient source having assimilable nitrogen to the rhizosphere at least at 1 ast as early as the time of planting.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 84/01741

I. CLASSIFI ATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC 3

A61K 37/00, A01C 1/06, A01C 1/00, C12N 5/00, C12N 5/02, C12N 1/00
C12 N 1/20, C12 R 1/01, C12R 1/41, C12 R 1/645

II. FIELDS SEARCHED

Minimum Documentation Searched 4

Classification System	Classification Symbols
US	424/93 47/57.6, 58 435/240, 241, 243, 253, 822, 878, 911

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched 5

Computor Search Biosis, Chemical Abstracts, Medline, Agricola under: Rhizobacteria, Pseudomanas, nematodes

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
A,N	Cox et al J of Bacteriology Vol 137(1) pp357-364 January 1979 "Isolation of an Iron-Binding compound from Pseudomonas averoginasa"	1-16
A,N	Jones et al Phytopathology Vol 64(12) October 1974 pp1507-1510" Susceptibitity of "Resistant" Tomato Cultivars to Fusarium Wilt"	1-16
A,N	Kloepper et al Current Microbiology Vol. 4 1980 pp317-320 "Pseudomonas Siderophores: A Mechanism Explaining Disease Suppressive Soils"	1-16
A,N	Teintze et al Biochemistry Vol 20 1981 pp6446-6457 "Structure of Ferric Pseudobactin, A Siderophore From a Plant Promoting Pseudomonas	1-16

* Special categories of cited documents: 15

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATI N

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
14 January 1985	17 JAN 1985
International Searching Authority 1	Signature of Authorized Officer 20
ISA/US	Robin Lyn Teskin

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